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16. ABSTRACT

Soil/litter microcosoms with "life support" systems were designed to maintain and monitor oxygen consumption, and community carbon dioxide and heat generation over extended time intervals. Mean coefficient of variation for oxygen comsumption and carbon dioxide generation rates of replicate processed systems was 6.5 percent. An initial adjustment period of rapid respiratory rates and bacterial growth was observed for 10-14 days after microcosm preparation and incubation at 20 C. Soil and litter moisture had marked and expected effects on community respiration. Conditions of high moisture favored bacteria; fungi were more prevalant under low moisture conditions.
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DESIGN AND PRELIMINARY RESULTS FROM SOIL/LITTER MICROCOSMS

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Soil/litter microcosms with "life support" systems were designed to maintain and monitor oxygen consumption, and community carbon dioxide and heat generation over extended time intervals. Mean coefficient of variation for oxygen consumption and carbon dioxide generation rates of replicate processed systems was 6.5 percent. An initial adjustment period of rapid respiratory rates and bacterial growth was observed for 10–14 days after microcosm preparation and incubation at 20°C. Soil and litter moisture had marked and expected effects on community respiration. Conditions of high moisture favored bacteria; fungi were more prevalent under low moisture conditions.

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INTRODUCTION

From biological intuition, laboratory experiments[1] and field measurements[2] it is apparent that environmental pollution affects soil decomposition processes. It has been estimated that 80–90 percent of the net primary production in terrestrial ecosystems is ultimately acted on by decomposer organisms[3–5] (Figure 1). Pollutants may alter the natural rate of decomposition in these systems with unknown, but crudely predictable, consequences. At low pollutant concentrations a stimulation of the decomposition rate could result in more rapid remineralization. At higher concentrations, lethal effects on decomposer organisms result in cessation of decomposition and resultant plant growth in the affected areas. Thus plant growth rates and standing stock of animals might be expected to vary as a function of soil decomposition rates.

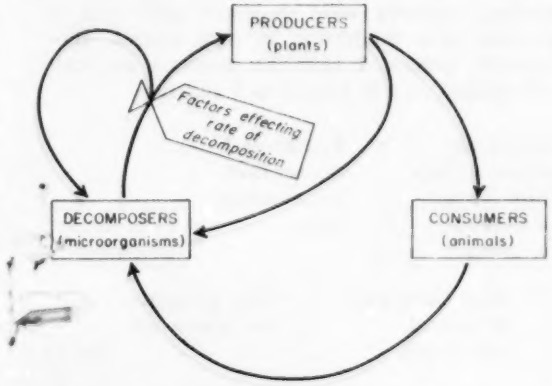

FIGURE 1 Simplified systems diagram of the organic matter cycle.

The study of pollutant stressants on decomposition in extramural laboratories such as field plots suffers from several shortcomings that make the use of laboratory microcosms attractive. Variation over short distances in natural or prepared soils confounds treatment effects analysis, often negating definitive results (e.g., Bond, Russell and Shimabuku, unpublished). To solve this problem large and numerous field plots must be used creating the problem of site decontamination after use of toxic and persistent pollutants. Even if an acceptable extramural site were found practical, this is only one of an almost infinite variety of soil types that would need to be studied and the use of many sites would be impractical. Soil/litter microcosms can and have been designed small enough to allow many treatments with replication to be performed in a single experiment and the contaminated soil/litter is

relatively easily disposed of. Further microcosm simulating a soil and/or litter system may be used to study nutrient or pollutant webs, pool sizes and transfer rates. Those data may be useful in construction of predictive computer simulation models of the system. Stotzky,[6] MacFayden,[7] McGarity, Gilmore and Bollen[8] and Pramer[9] discuss various aspects of soil microcosms.

To obviate the foregoing problems and to take advantage of the microcosm potential in the study of pollutant stress on decomposition processes, it was reasoned that if "identical" microcosms could be prepared that one might be able to more readily detect the effects of stressor treatment on the prototype. Stressants such as cadmium and selenium were chosen for these experiments because they are biologically active[10,11] elements that are found as soil contaminants in relatively high concentrations from several industrial and agricultural processes.[12,13] They were also chosen because of their handling convenience in aqueous solution.

This report gives preliminary results from a soil/litter microcosm system in which oxygen consumption, heat generation, and carbon dioxide produced was monitored over extended periods of time after cadmium and/or selenium treatment.

METHODS

Criteria followed during the design and development of the microcosm system were: (a) simplicity of design and operation, (b) minimization of cost, (c) minimization of instrumentation size, (d) operation for exended time periods, (e) ability to measure biological "integrator-indicators,"† (f) ability to measure "differentiated-indicators,"‡ and (g) to study soil/litter microbiology.

1. *System Design*

The system described here consists of a microcosm vessel inserted into a "life support" system (Figure 2). An earlier version of the system has been described.[1] The "life support" system consists of (a) reactor chamber, (b) temperature maintenance and heat generation monitoring module, (c) carbon dioxide production monitoring module, (d) pressure monitoring manometer, and (e) oxygen level maintenance and consumption monitoring module.

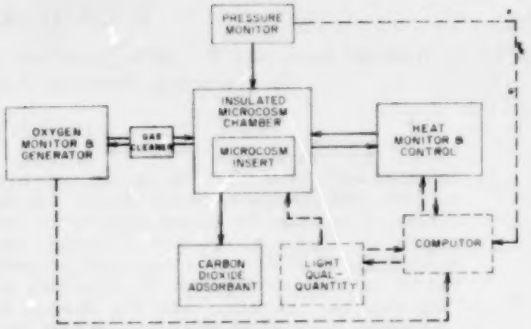

FIGURE 2 Systems diagram of the microcosm in its "life support" system. (Dashed line components are for future development.)

The reactor chamber module consists of a one liter Dewar flask large enough to accept the glass microcosm insert (600 ml glass beaker). The flasks were sealed with a foam stopper equipped with a small bore glass tube connected to the oxygen generator module, a thermal regulator,§ and wires to a heating resistor connected to the mercury bulb of the thermal regulator. The thermal regulator and heating resistor were electrically inter-connected to maintain the temperature of the reactor chamber above the "set point" of the thermal regulator. Reactor heat loss and resistor heating constants were measured and used to evaluate heat production in each reactor. Biologically generated heat (in calories) was calculated and used in Equations 1 and 1a:

biologically generated heat from the test microcosm (calories) = Test/control reactor heat loss constant ratio

× Heat Generated in control microcosm − Heat generated test microcosm (Eq. 1)

†Integrator-indicators were defined as the community heat, oxygen, and carbon dioxide measurements.

‡ Differentiated-indicators were defined as the organism groups responsible for the changes in the integrator-indicators.

§VWR, P.O. Box 3200, San Francisco, Calif., Catalog No. 61845-009.

$$\text{or } Q_{test} \qquad \frac{K_T}{K_C} \times (R_C \times t_C) - (R_T \times t_T) \qquad \text{(Eq. 1a)}$$

where Q_{test} = Biologically generated heat (calories) in the test reactor.

K_T = heat loss rate constants (cal/hr) for the test reactor.

K_C = heat loss rate constant (cal/hr) for the control reactor period.

R_C = heat production rate (cal/hr) constant for the control resistor.

R_T = heat reproduction rate (cal/hr) constant for the test resistor.

t_C = heat time (hrs) the control reactor resistor was heating.

t_T = heat time (hrs) the test reactor resistor was heating.

Carbon dioxide within the reactor was trapped in 10.0 ml of 0.60 *N* NaOH. Every 24 or 48 hours during an experiment the remaining NaOH in the vial was withdrawn through a permanently placed needle and valve in the reactor vessel top and titrated.[14,15] Data are presented as milliliters carbon dioxide generated during the trapping period.

In an earlier version of the system, oxygen level maintenance and monitoring was performed with a hydrolytic oxygen generator similar to that design by McGarity, *et. al.*[8] Oxygen consumed during microcosm respiration in the closed reactor system resulted in reduced internal pressure activating an electrolytic switch that causes oxygen to be delivered to the reactor. Hydrogen is stochiometrically generated at the cathode. One-half of the hydrogen volume generated is equal to the oxygen produced at the anode.

The system described here had a sodium sulfite/agar salt bridge embedded in frittered glass between the hydrogen and oxygen generating electrode, eliminating fluctuations in the electrolytic switch due to barometric pressure changes. A further modification in which oxygen will be generated at the anode and copper plated out at the cathode is now being tested. Ozone or other gases generated during the electrolytic reaction[16] is trapped in activated charcoal and nickel dibutyldithio-carbamate impregnated glasswool located in a U-tube between the oxygen electrode and reactor chamber.

Any gas leaks in the slightly positive pressure maintained system was monitored with a closed, water manometer.

2. *Experimental*

Four "life support" systems were constructed and the following five experiments performed to determine: (1) variation of respiratory rates and organismal growth between triplicate microcosms without pre-incubation, (2) variation of respiratory rates of four replicate microcosms after 14 days pre-incubation, (3) effect of soil moisture at 10, 30 and 60 percent of field water holding capacity on respiratory rates and organismal growth in triplicate microcosms, (4) effect of three levels of cadmium (0.0, 0.01, and 10.0 mg $CdCl_2$/kg dry soil) treatment on respiratory rates and organismal growth, and (5) effects of a combination of selenium and cadmium (10 ppm SeO_2 and 25 ppm $CdCl_2$) used singly and in combination on respiratory activity.

Microcosm vessels contained 150 grams of homogenized and sifted soil, overlain with 15 grams of sifted litter. Replicate microcosms were prepared by simultaneously sifting moisture-adjusted soil and litter components into a battery of microcosms until a predetermined weight of material was added. Compaction of the soil and litter was carried out by setting a lead brick on top of the material for one minute after each small addition of soil or litter.

Analyses for soil and litter moisture were determined gravimetrically on 105°C oven dried samples. Surface spread plate counts of total hetertrophic bacteria and microfungi were counted in triplicate on plates of 10-fold sterile phosphate buffer dilutions of agitated soil and litter samples on the following media: Acidified Potatoe Dextrose Agar[17] for fungi, and Bunt and Rovira's medium[18] for bacteria. Incubation was at room temperature for two weeks. Reactors were maintained at 20°C in a constant temperature room held at 17 to 18°C.

In the soil moisture experiment soil was moistened to the desired fraction of field water holding capacity prior to microcosm loading. Toxicants for the cadmium and selenium experiments were introduced into the microcosm by injecting an aqueous solution of $CdCl_2$ and SeO_2 into the soil and litter with a syringe. Inadvertently, water to be injected into the control reactor at the time of heavy metal addition to the selenium containing experiment was deleted.

RESULTS AND DISCUSSION

Results and discussion are organized for each experiment to present the integrator-indicator data (i.e., oxygen consumption and carbon dioxide production rates), and where data was collected, the differentiated-indicators (i.e., organism numbers).

Microcosms showed 10–14 days of comparatively high respiratory rates, thereafter remained relatively constant at the lower rate for at least another 400 hours (Figure 3). Variation of oxygen consumption and carbon dioxide generation rates changed with time among replicate microcosms, and between experiments (Table I). The coefficient of variation for these rates ranged from 9.9 percent to 3.7 percent with a mean of 6.5 percent for O_2 and CO_2 rates. The maximum absolute difference in rates of oxygen consumption and carbon dioxide generation was 600 percent between experiments.

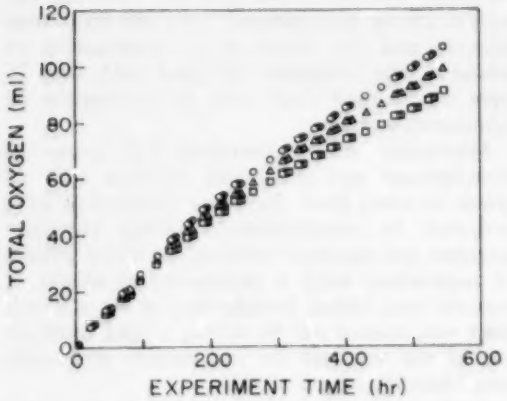

FIGURE 3 Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms.

The data indicate an initial rapid respiratory period of 10–14 days under our soil and incubation conditions before relatively constant respiration in the microcosms occurs. A partial explanation of the rapid initial rate is the concurrent rapid bacterial growth (Figure 4a). After 14 days pre-incubation the variation of O_2 and CO_2 rates within replicate sets of microcosms resulted in less than 10 percent coefficient variation; while between experiments variation is so great that large treatment effects must be observed to substantiate conclusions.

As expected, variation of soil and litter moisture content resulted in large changes in respiratory activity (Table I) and to a lesser extent microbial changes (Figure 4c, d). Compared to the driest soil/litter microcosm (i.e., 10 percent soil water holding capacity), respiration increased approximately 200 percent and 400 percent for soil and litter systems at 3 and 6 times the water content, respectively. This was reflected in increased abundance, and presumably metabolic activity, of the microflora under wetter conditions. In both soil and litter, the microbial quality appeared to shift toward the bacteria in wetter microcosms and toward the fungi in dryer microcosms. Soil moisture must be carefully controlled if inter-microcosm confounding is to be reduced for treatment comparison demonstrating that control of as many natural variables as possible is necessary if inter-microcosm variation is to be minimized. Controllable variables include: pH, organic matter quality and quantity, and soil particle size distribution.

Respiratory response, but not detectable microbial response (Figure 4e, f) varied with amount of cadmium added to microcosms (Figure 5) and with time of observation. At low cadmium concentrations (0.01 ppm $CdCl_2$), a transient stimulatory effect (increased respiratory rate) was noted for the initial 200 hours after amendment, followed by recovery to control rates. Respiration at high cadmium concentrations (10 ppm $CdCl_2$) equaled that of the control for approximately 300 hours after amendment; thereafter, respiration decreased by 40 percent compared to the control. The mechanism of the dramatic loss of activity at high cadmium levels is unknown. There was no decrease in numbers of culturable soil or litter fungi or bacteria, although there was a relative increase in oxygen consumed over carbon dioxide produced (Figure 6) suggesting uncoupling of oxidative phosphoralation; a common heavy metal effect.[10] Stimulation at low levels and inhibition at higher levels by heavy metals is a common microbial response to heavy metals: oligodynamic action.[19]

In the combined selenium/cadmium experiment, as with the high cadmium at three levels experiment, there was a delay of several hundred hours before an inhibitory effect on oxygen con-

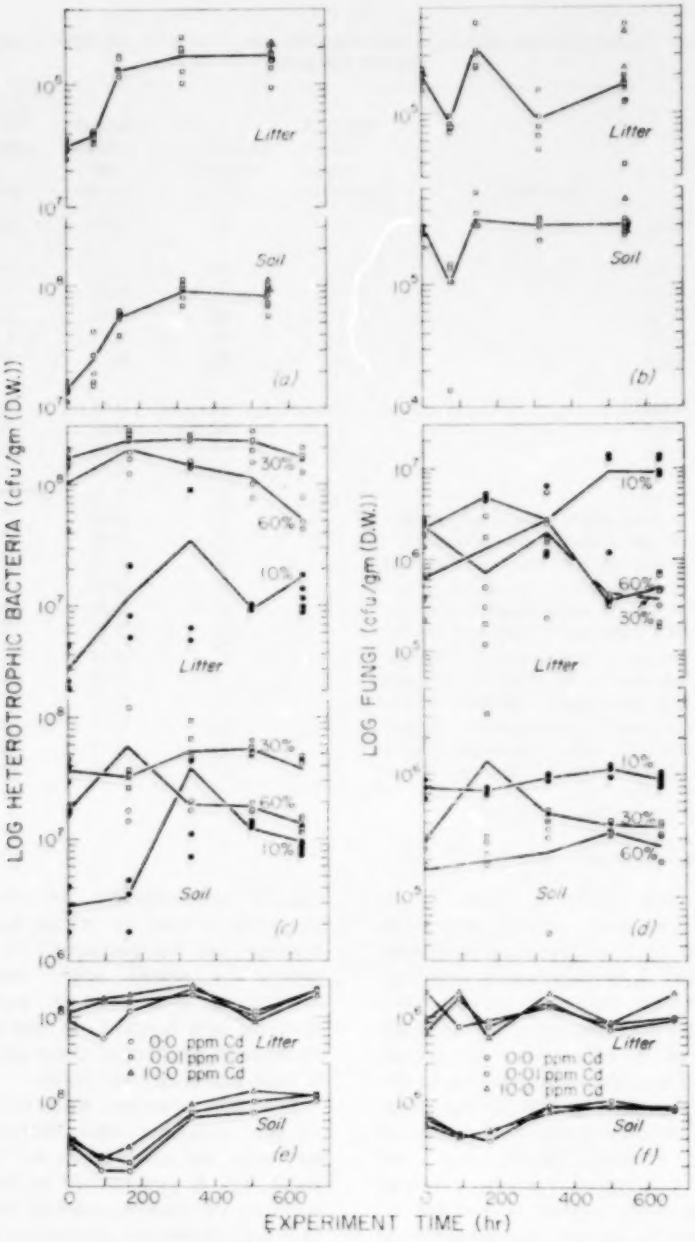

FIGURE 4 Graph of the hetertrophic bacterial and fungal propagules (cfu=colony forming units) in soil and litter in three "identically" processed microcosms (a and b); 3 "identically" processed microcosms incubated at 10, 30, and 60 percent field water holding capacity respectively (c and d); and three "identically" processed microcosms injected with distilled water 0.0, 0.01, and 10.0 ppm final mean concentration $CdCl_2$ respectively (e and f).

TABLE I

Tabulation of oxygen consumption and carbon dioxide production rates during the experiments and incubation time intervals indicated.

Experiment	Replicate	Treatment	10 to 14 days pre-incubation	Incubation interval (days)	Oxygen uptake rate (ml/hr)	Carbon dioxide generation rate (ml/hr)	Coefficient of variation (%)
1	1	None	—	14	0.25	0.40	[a]CV_{O_2} = 5.03
1	2	None	—	14	0.24	0.36	[a]CV_{CO_2} = 6.65
1	3	None	—	14	0.22	0.36	
1	1	None	+	10	0.16	0.19	CV_{O_2} = 3.83
1	2	None	+	10	0.15	0.18	CV_{CO_2} = 9.93
1	3	None	+	10	0.13	0.18	
2	1	None	+	14	0.68	0.67	CV_{O_2} = 9.81
2	2	None	+	14	0.67	0.72	CV_{CO_2} = 3.67
2	3	None	+	14	0.61	0.64	
2	4	None	+	14	0.55	0.67	
2	NA[b]	None	+	27	0.31[c]	NA	NA
2	NA	25 ppm Added Cadmium Chloride	+	27	0.43	NA	NA
2	NA	10 ppm Added Selenium Dioxide	+	27	0.41	NA	NA
2	NA	25 ppm Added $CdCl_2$ + 10 ppm SeO_2	+	27	0.25	NA	NA
3	NA	10% Field Water Holding Capacity	+	12	0.03	0.05	NA
3	NA	30% Field Water Holding Capacity	+	12	0.08	0.12	NA
3	NA	60% Field Water Holding Capacity	+	12	0.12	0.19	NA
4	NA	0.0 ppm Added Cadmium Chloride	+	7	0.14	0.25	NA
4	NA	0.01 ppm Added Cadmium Chloride	+	7	0.18	0.26	NA
4	NA	10.0 ppm Added Cadmium Chloride	+	7	0.13	0.21	NA
4	NA	0.0 ppm Added Cadmium Chloride	+	24	0.14	0.16	NA
4	NA	0.01 ppm Added Cadmium Chloride	+	24	0.14	0.14	NA
4	NA	10.0 ppm Added Cadmium Chloride	+	24	0.08	0.10	NA

[a] Mean CV_{O_2} = 6.22%, Mean CV_{CO_2} = 6.75%.
[b] NA = Not available or applicable.
[c] Control water injection inadvertantly deleted.

sumption was observed (Table I, Figure 7). In the selenium-only treatment, effects were seen almost immediately after pollutant amendment. The interaction effect of selenium and cadmium on oxygen consumption rate was greater than either toxicant alone and amounted to approximately 55 percent of the effect when the metals were added singly. The reduced respiration in the control reactor is presumably due to the inadvertant deletion of distilled water at the time of pollutant addition. Previous results (e.g., see Figure 5) indicate respiration remains constant with water addition to the control microcosm.

Additional methods to measure treatment effects on biological components in the soil/litter systems, through gnotobiotic addition of cultured microarthropods, nematodes, bacteria and fungi has been initiated. The bacterial and fungal components to be added to the microcosms would be typical representatives of clusters[20-22] of most prevalent isolets for which fluorescent antibody systems can be prepared[23] or specific selection procedures would allow their enumeration.[24] Studies in progress will measure changes in bacterial and fungal flora and to a limited extent changes in microarthropods and nematodes, using optical scanning procedures.

Fourteen systems, with modifications of the oxygen generator and thermostat modules to automate the microcosms for computer management, will be operational in the future. Response surfaces of multiparameters will be determined in 3 x 3 treatment experiments using nine of these systems. Four systems will be used in multi-single parameter experiments. One system will be used in design development research.

Data from the microcosm systems will be used in response structure computer simulation

models[25,26] and later in more comprehensive compartment models.[27–29] Response structure measurements will include both integrator as well as differentiated variables as affected by natural conditions such as soil moisture, temperature, organic quality and quantity, and particle size quality and quantity. Effects of pollutant materials such as heavy metals or air pollutants will also be estimated. These experiments are anticipated to evaluate transfer through food webs, estimate organizmal pool sizes, and provide information on toxicant kinetics between pools.

The goal of his research is to develop and use the knowledge gained to predict the consequence of pollutant addition on soil decomposition processes.

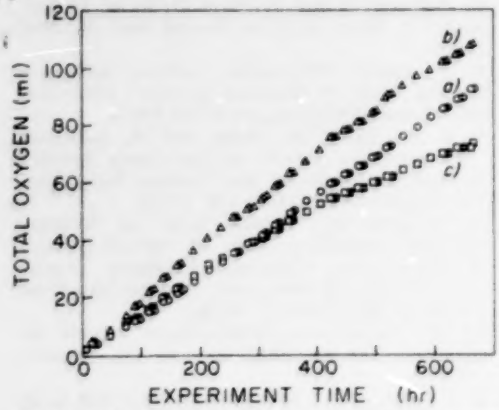

FIGURE 5 Accumulated oxygen consumed through time in three identically processed coniferous forest microcosms treated to final mean concentration of (a) 0.0, (b) 0.01, (c) 10.0 ppm cadmium chloride.

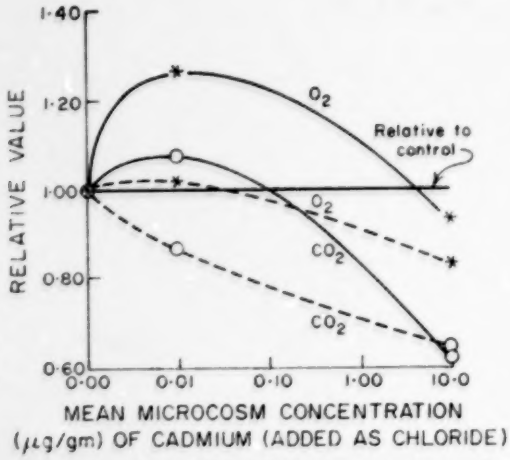

FIGURE 6 Graph of the relative oxygen consumption (*), and carbon dioxide generation (o) rate values for the initial 200 (—) and subsequent (---) hours after cadmium addition (mean microcosm concentration) to douglas fir trees soil/litter microcosms.

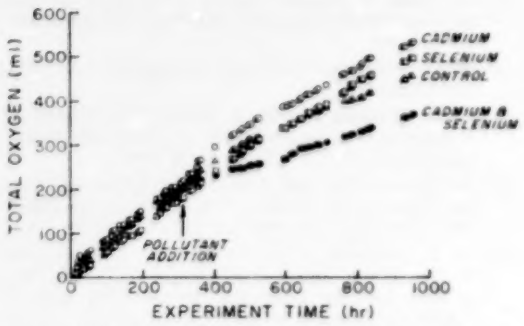

FIGURE 7 Accumulated oxygen consumed through time in four "identically" processed coniferous forest microcosm treated to a final mean concentration of nothing for the control, 25 ppm cadmium chloride and/or 10 ppm selenium oxide.

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